Amperometric Urea Biosensor Using Polypyrrole with Different Dopants.

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The urea concentration in serum or urine, is an indicator of kidney diseases, as well diabetes, and analysis in clinical laboratories is very frequent. However, the urea quantification uses conventional methods, such as spectrophotometric with indophenol[1]. In this way, the development of new routes to urea determination is very important, and the biosensors are a very good alternative that involves a low cost, fast response, and others qualities.

In a urea biosensor the enzyme urease, which catalyses the hydrolysis of urea to ammonia and carbonate, can be immobilized into different transducers, such as conducting polymer. It is well know that polypyrrole films is the most widely used conducting polymer to the construction of sensors and biosensors, once their conductivity and electroactivity do not strongly depend on pH of the electrolyte. In previous works it was demonstrated the efficiency of polypyrrole as a mediator to the ammonia detection. And its response could be improved using different dopants into the film preparation[2].

The aim of the present work remains improving amperometric urea determination. The biosensors were elaborated by the enzyme immobilization in DBSA or chloride doped polypyrrole film in three different methods. The first one, consist in the enzyme immobilization with the electropolymerization of pyrrole. The second method was based on cross linking the enzyme in to the polymer using glutaraldehyde. And the last one the enzyme was immobilized by physical adsorption followed by an entrapment using cellulose acetate.

For each method applied, different parameters were investigated, such as different amount of deposited polymer, controlled by the deposition charge, several quantities of enzyme, and others. Each distinct method produces a biosensor with very particular advantages, like linear response in a wide range of urea concentration, for enzyme immobilized directly to the electropolymerization; or less signal to the interference caused by ascorbate and ureate and a longer live time for biosensors prepared by the entrapment of the enzyme with cellulose acetate in polypyrrole.

For electrodes with enzyme immobilized during the monomer polymerization, the amount of polymer formed is limited, and in this way, also the amounts of enzyme. An augmentation in the polymer quantity deposited leads to a sensitivity increment and also to bigger values of current densities. No good response was observed by DBSA doped polypyrrole films, related to the fact of competition between DBSA anions and enzyme as counter ions during the electropolymerization.

Enzyme immobilization using glutaraldehyde provides a biosensor with satisfactory sensitivity to urea (0.05 up to 0.40 mA cm^{-2} M^{-1} dependent on the film thickness and polymeric dopant). But some problems were related with the glutaraldehyde use like as a decrease of polymer adhesion to the electrode and a decrease of the amperometric response. The optimal concentration of glutaraldehyde formed was c.a. 1.5 %.

The cellulose acetate produces a very good film on top of the electrode with PPy deposited and the enzyme adsorbed. The response were very proficient with a sensitivity between 0.10 up to 0.20 mA cm^{-2} M^{-1}.

The life time experiments showed the best results to enzyme immobilized with cellulose acetate with a drop to 80 % of initial value after 15 days. These electrodes also exhibited the lowest response to interference such as ascorbic and uric acid.

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References

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